REMARKS

Reconsideration and allowance are respectfully requested.

Claims 1-35 and 39-46 are pending. Applicants acknowledge the statement on page 2 of the Action that upon finding a generic claim allowable, examination will be extended to include methods for non-animal organisms and cells.

The claim amendments are supported by the original disclosure and, thus, no new matter has been added. For example, the specification describes a ribonucleotide sequence comprising at least 25 bases which correspond to the target gene on page 6, lines 24-26, and page 11, lines 27-28; the formation of a double-stranded RNA structure under annealing conditions on page 28, line 30, to page 29, line 5. But if the Examiner should disagree, she is respectfully requested to point out the challenged limitation with particularity in the next Office Action so that support may be cited in response.

New claims 43-46 have been added to provide alternatives for claiming the invention. In particular, claims 43-44 explicitly recite that ribozymes are not within the scope of those claims. Ribozymes were known in the prior art and are characterized in that they are autocatalytic without the need for protein cofactors. In contrast, RNA interference requires protein cofactors. Biochemical assays using drosophila lysates and wheat germ extracts show that the former recapitulates dsRNA-specific degradation whereas the latter lacks required components of the reaction (see page 3195 of Tuschl et al., Genes Dev., 13:3191-3197, 1999). Moreover, genes encoding protein cofactors required for RNA interference have been identified (e.g., Tabara et al., Cell, 99:123-132, 1999).

Thus, although ribozymes are not explicitly discussed in the specification, it is implicit in Applicants' description of the invention that RNA interference does not involve the autocatalytic activity of a ribozyme. For example, specific approaches to inhibition of gene expression like antisense, triple helix, and co-suppression are explicitly contrasted to RNA interference in the Background of the Invention section. And the discussion concludes, "The present invention avoids the disadvantages of the previously-described methods for genetic interference" (page 5, lines 27-28, of the specification). Therefore, it would be clear to persons skilled in the art that Appli-

cants do not include a prior art method like ribozyme-mediated inhibition of gene expression within the scope of their claimed invention.

Attached is a Form PTO-1449 listing the enclosed documents. This supplemental information disclosure statement is intended to be in full compliance with the rules, but should the Examiner find any part of its required content to have been omitted, prompt notice to that effect is earnestly solicited, along with additional time under 37 CFR § 1.97(f), to enable Applicants to comply fully. The Rule 17(p) fee required under 37 CFR § 1.97(c) in lieu of certification is filed herewith. Consideration of the foregoing and enclosures, as well as the return of a copy of the Form PTO-1449 with the Examiner's initials per MPEP § 609, are earnestly solicited.

35 U.S.C. § 112 - Definiteness

Claims 1-6, 8-23, 25-35 and 39-40 were rejected under Section 112, second paragraph, as being allegedly "indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." Applicants traverse for the following reasons.

The term "portion" has been deleted from claims 1, 22 and 39-40 because it is not required for patentability. The phrase "identical nucleotide sequence" has also been deleted because it is not required for patentability. The first and second ribonucleotide sequences of the double-stranded structure is described as comprised of at least 25 bases which correspond or are complementary to a nucleotide sequence of the target gene, respectively, (claims 1 and 39-40) to address the Examiner's objection that thymidine and uracil are not identical.

Moreover, the ribonucleic acid strands of the double-stranded structure are described as each being able to specifically hybridize to the target gene (claim 22): i.e., one RNA strand hybridizing to the coding sequence of the target gene and the other RNA strand hybridizing to the complementary sequence of the target gene.

Furthermore, the Examiner's suggestion to provide a clearer description of the double-stranded RNA has also been adopted. In particular, RNA of the invention is now described as comprising at least 25 bases per strand (e.g., claims 1, 22, 39-40 and 43-44) and/or resulting from stably annealing of the two strands (e.g., claims

1, 39-41 and 43-46). Exemplary annealing conditions are described on page 28, line 30, to page 29, line 5, of the specification.

The phrase "the identical nucleotide sequence" has been deleted from claim 28 because it is not required for patentability. Claim 28 has been amended to be consistent with amended claim 22.

The Examiner's suggestion for clarifying claim 33 has been adopted.

Antecedent basis of "the expression construct" has been corrected by making claim 35 depend from claim 34. This was a typographical error.

Claim 39 has been amended to explicitly recite that the RNA is a component of the kit.

Applicants request withdrawal of this claim rejection made under Section 112, second paragraph, because the pending claims are clear and definite.

35 U.S.C. § 112 – Enablement

The Patent Office has the initial burden to question the enablement provided for the claimed invention. M.P.E.P. § 2164.04, and the cases cited therein. It is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. *In re Marzocchi*, 169 USPQ 367, 370 (C.C.P.A. 1971). Specific technical reasons are always required. See M.P.E.P. § 2164.04.

Claims 1-6, 10-23, 27-35 and 40 were rejected under Section 112, first paragraph, because the specification, while being enabling for method of inhibiting expression of a target gene using a double stranded RNA in nematodes or in vitro, allegedly "does not reasonably provide enablement for methods of inhibiting expression of a target gene using a double stranded RNA in any organism in vivo (whole organism)." Applicants traverse.

An enablement rejection must be based on factual findings. See *Marzocchi* at 370. But the arguments on which this enablement rejection is based do not provide the specific technical reasons from which a person skilled in the art would conclude that the claimed invention would require undue experimentation for its practice.

Firstly, references discussing the problems that have been encountered in gene therapy are cited on pages 7-8 of the Action. The Examiner appears to be concerned that insufficient amounts of RNA would be delivered to the cell or made therein by an expression vector. Such concerns are not applicable to the Applicants' invention. In many cases, RNA-mediated interference is transient and observation of the effects of inhibiting gene expression in a subpopulation of cells is adequate for applications such as functional genomic studies (see also pages 20, lines 9-15, of the specification). Direct methods of introducing the RNA into a cell such as micro-injection and electroporation can be used. Moreover, the double-stranded structure of the RNA stabilizes the molecule against degradation (see page 4, lines 19-20, of the specification). Furthermore, the dosage of RNA that would be required for effective sequence-specific inhibition is much lower than that required for gene therapy or antisense inhibition (see page 4, lines 21-24, of the specification).

Secondly, references describing the effect of RNA-mediated interference are discussed on pages 8-9 of the Action. The Examiner appears to be concerned that the effects in mammals are unpredictable. As noted above, transient inhibition of gene expression is useful and would be expected when RNA per se is used instead of an expression vector transcribing the RNA. Specific RNA-mediated interference with gene expression has been demonstrated in vivo for mouse oocytes (Svoboda et al., 2000) and mouse embryos (Wianny et al., 2000). The Examiner states that "inhibition by double stranded RNA is transient, and function is regained after multiple cell divisions." This is correct. But it is also the expected result because dsRNA per se was microinjected and cell division during embryogenesis causes dilution (see last two paragraphs on page 74 of Wianny et al.). Such results described on page 23, lines 24-28, of the specification is useful to show that the effect is gene specific and that the double-stranded RNA is effective when present at only a few molecules per cell. This confirms that large amounts of expression vector or high doses of RNA per se is not necessary for effective inhibition.

As discussed previously in Applicants' Amendment dated December 2, 2000, results in zebrafish have been equivocal. Wargelius et al. (1999) and Li et al. (2000) describe successful results in zebrafish in vivo. Xenopus, another vertebrate animal, has also been used to demonstrate inhibition of gene expression in vivo by double-stranded RNA (Nakano et al., 2000). Oates et al. (2000) is cited by the Examiner for

showing the unpredictability of the invention. The authors describe their results as nonspecific inhibition of gene expression. But as Oates et al. admit on page 26, "It is possible that a gene-specific effect might be produced, but be masked by the non-specific effects seen at dsRNA concentrations sufficient to perturb morphology or deplete endogenous mRNAs." They hypothesize in the paragraph bridging pages 26-27 that the specific effects of dsRNA may be masked by the immune response induced by double-stranded RNA. This is one explanation for their results, but it should also be noted that Oates et al. were unsuccessful in their attempt to reproduce the positive results of Li et al. (paragraph bridging pages 24-25). Therefore, it would be unreasonable to ignore the positive results cited above that were obtained in vertebrate organisms in vivo in favor of the negative result of Oates et al. (2000).

Thus, the evidence of record demonstrates that despite scattered negative reports in the literature (e.g., Oates et al., 2000), the weight of the evidence shows that double-stranded RNA causes inhibition of gene expression in a remarkably wide variety of species and cell types for plants, single-cell organisms, invertebrate and vertebrate animals, and cultured cells. The interferon response is a complicating factor in organisms with immune systems, but the prior art teaches that such nonspecific effects are not observed using short lengths of RNA. This complication was recognized by Applicants, but it did not appear to be relevant in their examples (see page 24, lines 9-14, of the specification). Of course, in vertebrate animals which usually have an immune system or panic response, nonspecific effects that mask the gene-specific effects of the invention may be avoided by using conditions (e.g., low concentrations or short lengths) which do not induce the nonspecific effects that inhibit a broad spectrum of genes.

Recent publications that are submitted herewith in an information disclosure statement (e.g., Elbashir et al., 2001; Bass, 2001; Caplen et al., 2001), illustrate that shorter double-stranded RNA will cause gene-specific inhibition and avoid the complications of inducing the immune/panic response. Therefore, the teachings of the prior art of how to avoid inducing the interferon response and the teaching in Applicants' specification that even short RNA (i.e., at least 25 bases) could be effective avoid the concern that undue experimentation would be required "to overcome the effects of dsRNA induced immune response" as stated on page 9 of the Action.

Finally, it should be noted that claim 40 is drawn to inhibiting gene expression in a cell of an invertebrate animal. Given the abundant evidence that undue experimentation was not required to practice the claimed invention in invertebrate animals, clarification is respectfully requested for why claim 40 is included in this rejection.

Applicants request withdrawal of this enablement rejections because undue experimentation would not be required for a person of skill in the art to make and use the claimed invention. But to advance prosecution in this matter, the claims now recite that the cell or organism is susceptible to inhibiting gene expression by RNA interference or inhibition of gene expression by dsRNA.

35 U.S.C. § 102 - Novelty

A claim is anticipated only if each and every limitation as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. v. Union Oil Co. of Calif.*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). The identical invention must be shown in as complete detail as is contained in the claim. See *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

Claims 1-6, 8-11, 13, 17-18, 22-23, 25-26, 28, 30-31 and 40 were rejected under Section 102(b) as being allegedly anticipated by Agrawal et al. Applicants traverse because all limitations of the claimed invention are not taught by the cited reference.

Agrawal et al. (WO 94/01550) discloses self-stabilized antisense oligonucleotides. But the oligonucleotides disclosed by Agrawal et al. are not taught to contain a double-stranded RNA structure with first and second ribonucleotide sequences as recited in Applicants' claims. This reference is directed to using a double-stranded region to make a nucleic acid resistant to degradation. Thus, the double-stranded structure of Agrawal et al. is not integral to the antisense function of the oligonucleotides. Instead, that structure simply makes the nucleic acid resistant to degradation.

The self-stabilized antisense oligonucleotides are described by Agrawal et al. as having two regions: a target hybridizing region and a self-complementary region (page 5, lines 13-17). The oligonucleotides may include polymers of ribonucleotides (page 8, lines 10-12). The cited reference neither teaches nor suggests that nucleotide sequences in the self-complementary region be designed to correspond or be

complementary to a nucleotide sequence of the target gene. Instead, Agrawal et al. teach the use of oligonucleotides in which a single-stranded region is the nucleotide sequence responsible for interacting with the molecule's target.

Exemplifying this point, the thermodynamic argument made by Agrawal et al. on page 9, lines 3-18, and illustrated in Figure 3 of the reference would suggest that such overlaps between the target sequence and self-complementary region would be short to allow the disruption and replacement to occur.

This distinction between the double-stranded structure and the functional part of the nucleic acid in the antisense oligonucleotides of Agrawal et al. is unlike Applicants' invention. In particular, the claims recite that RNA of the invention "comprises" or "consists essentially of" the double-stranded structure. Therefore, the presence of a functional single-stranded structure in the antisense oligonucleotides of Agrawal et al. does not anticipate the claimed invention.

Finally, the chemical requirements for RNA of the claimed invention are significantly different from the antisense oligonucleotides of Agrawal et al. Parrish et al. (Mol. Cell, 6:1077-1087, 2000; see, for example, Fig. 5) demonstrate that a specific chemical structure is required for RNA interference: natural or modified ribonucleotide bases for both strands of the double-stranded RNA structure are needed. Agrawal et al. do not teach or suggest any chemical specificity for their invention and, in particular, do not describe any preference for RNA over DNA in the composition of their antisense oligonucleotides.

Agrawal et al. do not anticipate the claimed invention because all limitations of independent claim 1, 22 or 40 are not found in the cited reference. Moreover, those claims depending from the independent claims are also not anticipated by the reference because the limitations of claim 1, 22 or 40 are incorporated in the dependent claims. See *In re McCarn* 101 USPQ 411, 413 (C.C.P.A. 1954).

Claims 1, 4-6, 11, 13, 21-23, 31 and 34 were rejected under Section 102(e) as being allegedly anticipated by Draper et al. Applicants traverse because all limitations of the claimed invention are not taught by the cited reference.

Draper et al. (U.S. Patent No. 5,972,704) discloses ribozymes or enzymatic RNA molecules which cleave HIV-1 transcripts. Such molecules are defined at col. 4, lines 18-24, as having an enzymatic activity which is active to specifically cleave

target transcripts. But the RNA of the claimed invention do not include the characteristic features of ribozymes (e.g., hammerhead and hairpin motifs) and cleavage of target transcripts by the invention does not occur without protein cofactors. Therefore, inhibition of gene expression by ribozyme-mediated cleavage of target transcripts does not anticipate the claimed invention.

Independent claims 1 and 22 have been amended to clarify that RNA of the invention comprises a structure which is distinct from ribozymes. The cited reference does not anticipate the claimed invention because the dsRNA structures described by Applicants have been demonstrated to lack a ribozyme's autocatalytic activity (i.e., cleavage of target RNA in the absence of protein cofactors) and because RNA interference has been shown to require protein cofactors.

Draper et al. does not anticipate the claimed invention because all limitations of any independent claim are not found in the cited reference. Moreover, those claims depending from the independent claims are also not anticipated by the reference because the limitations of the independent claims are incorporated in the dependent claims. See *In re McCarn* 101 USPQ 411, 413 (C.C.P.A. 1954).

For reasons of record, Applicants respectfully submit that the claim rejections made under Section 102 should be withdrawn.

35 U.S.C. § 103 – Nonobviousness

To establish a case of *prima facie* obviousness, all claim limitations must be taught or suggested by the prior art. See M.P.E.P. § 2143.03.

Claim 39 was rejected under Section 103(a) as being allegedly unpatentable over Agrawal et al. Applicants traverse because Agrawal et al. do not teach RNA in accordance with the claimed invention. The cited reference neither teaches nor suggests an RNA having a double-stranded structure with first and second ribonucleotide sequences each comprising at least 25 bases which either correspond to or are complementary to a nucleotide sequence of a target gene. Nor does it teach or suggest that those first and second ribonucleotide sequences stably anneal to each to form the double-stranded structure.

Applicants respectfully submit that the claim rejection made under Section 103 should be withdrawn because all limitations of claim 39 are neither taught nor suggested by Agrawal et al.

Conclusion

Having responded to all pending objections and rejections in the Office Action (Paper No. 17), Applicants urge that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is needed.

Respectfully submitted,

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